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THE SWITCHOVER FROM VIRGIN TO MATED BEHAVIOR IN FEMALE CECROPIA MOTHS: THE ROLE OF THE BURSA COPULATRIX

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In their reproductive behavior the non-feeding giant silkmoths are well adapted to a short life span of 7 to 10 days. The female emerges with almost a full complement of mature eggs (Telfer and Rutberg, 1960) and soon thereafter begins to release sex pheromone ("call") in response to specific environmental cues (Riddiford and Williams, 1971). As a virgin the female lays no eggs for at least the first 3 days after emergence. Beyond this time eggs are laid at a low rate of approximately 7% of her mature eggs per day (Truman and Riddiford, 1971). Mating causes an abrupt switch in the behavior of the female: she ceases to "call" and begins to lay eggs at a greatly increased rate. Mated females of *Hyalophora cecropia* oviposit an average of 36% of their mature eggs on the first night and thereafter lay approximately 35% of their remaining eggs each succeeding night (Truman and Riddiford, 1971).

Intrinsic neurosecretory cells of the corpora cardiaca were found to control both the "calling" and the oviposition behavior of these moths (Riddiford and Williams, 1971; Truman and Riddiford, 1971). Yet the question remained as to how mating stimulated the corpora cardiaca to release the presumed hormone which caused the change to mated behavior. The failure of matings to castrated males to trigger the increase in oviposition rate (Truman and Riddiford, 1971) indicated that sperm were necessary for the switchover in behavior. The present study was done to determine the type of link between the presence of sperm in the genital tract of the female and the neuroendocrine system.

Materials and Methods

Experimental animals

Pupae of *Hyalophora cecropia* were purchased from dealers or reared outdoors on netted trees (Telfer, 1967). These pupae were stored at 5° C for at least 12 weeks prior to use.

Castrations

The pupal testes were removed from chilled male diapausing pupae as described by Truman and Riddiford (1971). These castrated pupae formed apparently normal moths which mated and produced sterile but well-formed spermatophores.

Organ implantations

The bursa copulatrix was excised just proximal to the chitinized ductus bursae and placed into Ringer's (Ephrussi and Beadle, 1936). The adhering fat body and, in the case of mated females, the spermatophore were removed. The empty bursa was then rinsed in fresh Ringer's and implanted into a lightly anesthetized (10 minutes under CO_2) two-day-old virgin Cecropia female through a slit in the dorsal part of the second abdominal segment. A few crystals of a 1 : 1 mixture of phenylthiourea and streptomycin (Williams, 1959) were placed in the wound, which was then closed by a hemostat. One to 2 hours later, the hemostat was removed and melted paraffin was used to cover the wound site.

The spermatheca including the lagena was removed to Ringer's. Most of the sperm were removed from those from mated females, although it was later found that the presence of sperm did not alter the results obtained. Implants were performed as with the bursa.

Hemolymph injections

The blood was expressed from a middorsal slit in the sixth abdominal segment by pressing down steadily on the first abdominal segment. The blood was removed with a Pasteur pipet to a microscope slide. A few crystals of a 1 : 1 mixture of phenylthiourea and streptomycin were added to prevent darkening. The blood was then taken up into an 100 μl Hamilton syringe with a fixed 30-gauge needle. Fifty μl were injected into each recipient two-day-old female between the first and second ventral abdominal segment just below the spiracle. The wound was sealed with melted paraffin.

Collection of eggs

After organ implantation or blood injection, the female moth was placed in a large paper bag. The number of eggs laid each day was then counted. After death, the female was dissected under Ringer's solution and the number of mature chorionated eggs remaining in the ovarioles counted. Thus, the total number of mature eggs was determined and used as a base for the cumulative percentage laid per day.

Only host females which lived for at least four days after the experiment were included in the data. Females that died in two to three days tended to produce a sudden burst of oviposition the day of their death, irrespective of the treatment. Similarly, females which laid no eggs after treatment were discarded, although some lived as long as two weeks.

H^3-inulin

Ten μl of water containing 0.36 μg inulin-methoxy-H^3 (New England Nuclear, 139 μCi/mg) were injected into the dorsal thorax of two-day-old virgin female Cecropia moths. Twenty-two hours later the females were bled and 10 μl of blood was counted in a Tracerlab liquid scintillation counter.

Results

Implantation of spermathecae

Since matings to castrate males did not result in a mated oviposition response (Truman and Riddiford, 1971), it seemed likely that the presence of sperm in the

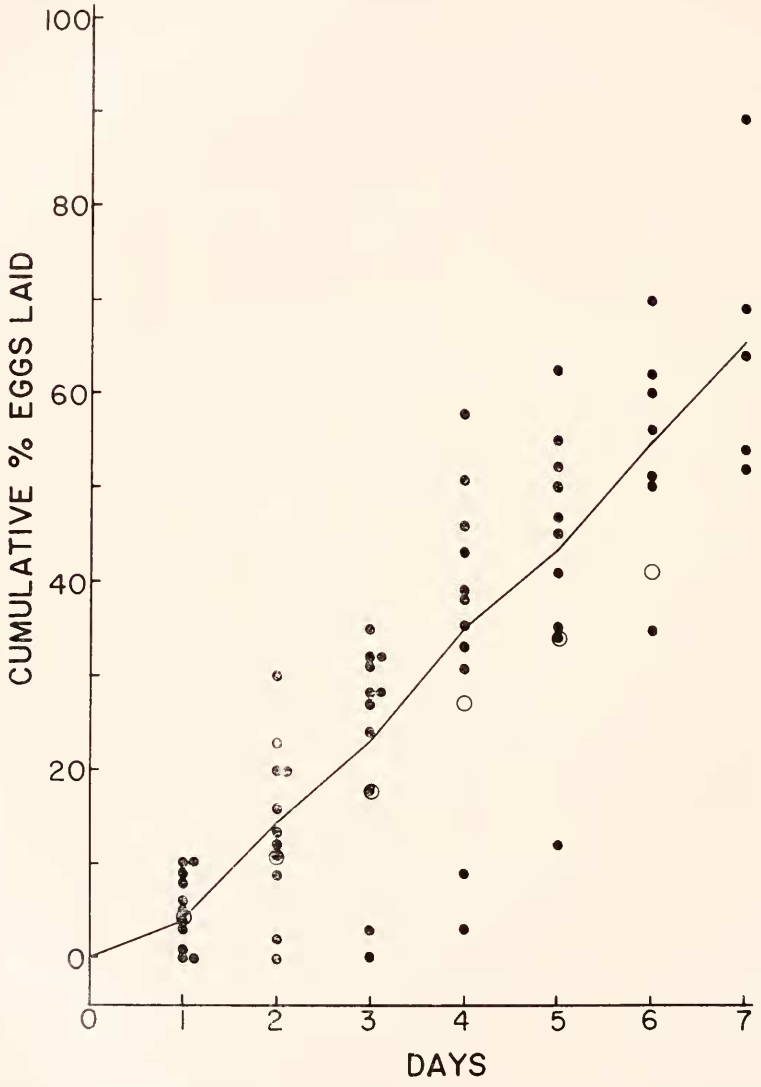

Figure 1. The oviposition pattern of virgin Cecropia females, each of which has received an implant of a spermatheca from a mated female. The abscissa refers to days after implantation. The line is drawn through the average of the closed circles. The open circles represent the average cumulative percentage of eggs laid by normal, intact virgin females, beginning on day 3 of life (Truman and Riddiford, 1971).

spermatheca was the trigger for the switchover from a virgin to a mated oviposition rate. Thus, 12 females were mated. The spermatheca was removed the morning after mating was completed and was implanted into a virgin female. Figure 1 shows that these females laid eggs in a pattern which was essentially identical to that displayed by virgin females.

Implants of spermathecae from 4 two-day-old virgin females also gave the same pattern.

Implantation of the bursa copulatrix from mated females

Cecropia mate in the early morning and remain *in copulo* throughout the day until the onset of darkness at which time the female begins laying eggs. Figure 2A shows that implantation of the empty bursa copulatrix from a mated female into a virgin female caused the switchover from the virgin to the mated oviposition pattern. Five of the 17 bursae were removed immediately after the termination of mating before the female began laying eggs; 10 on the next morning; and 2 were removed on the following day. No detectable difference in the oviposition rate

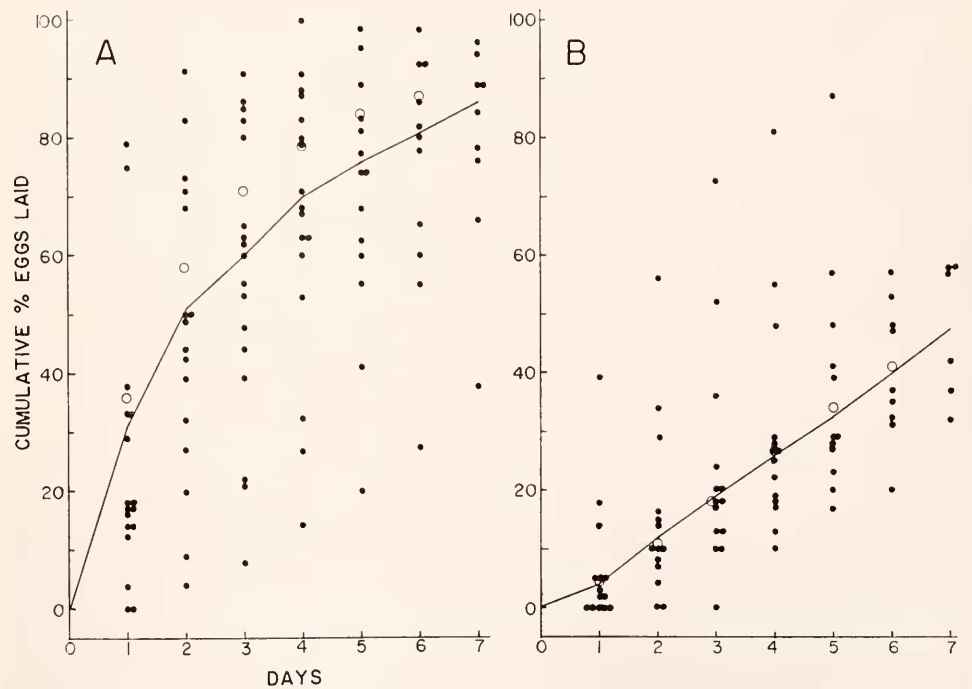

FIGURE 2. The oviposition patterns of virgin female Cecropia, each of which has received an implant of a bursa copulatrix. The abscissa refers to days after implantation. The lines are drawn through the average of the closed circles excluding the lowest set in 2A and the highest set in 2B: (A). mated bursa implanted; (B.) virgin bursa implanted. The open circles represent the average cumulative percentage of eggs laid by normal, intact mated and virgin females respectively (Truman and Riddiford, 1971).

was noted among these groups. All females except two laid eggs in the mated oviposition pattern.

Figure 2B indicates that the trauma involved in the implantation of the large bursa was not responsible for the increased oviposition rate. When bursae were removed from 14 two-day-old virgin Cecropia and each implanted into a virgin female, the oviposition pattern obtained was, with the exception of one female, always that of a virgin female. Consequently, one may conclude that bursae from mated females emit a blood-borne factor which effects the switchover in oviposition behavior after mating.

Implantation of the bursa copulatrix from females mated to castrate males

Eleven females were mated to castrate males. On the morning following completion of mating the bursa was excised and the spermatophore removed. These bursae when implanted into virgin females failed to cause a switchover in oviposition pattern (Figure 3). The per cent of eggs laid was only slightly increased over that seen when virgin bursae were implanted. Therefore, the oviposition pattern remained essentially that of a virgin female. Since these bursae had contained a well-formed but sterile spermatophore, apparently an interaction of the bursa with the sperm is necessary to trigger the release of the bursa factor.

Injection of hemolymph from mated females

Since both the corpora cardiaca (Truman and Riddiford, 1971) and the bursa copulatrix of mated females presumably release hormones into the blood, the hemolymph from these females should be able to stimulate oviposition. Injected hemolymph proved somewhat toxic such that death often occurred in 12–48 hours. All females which lived at least 3 days after the injection were included. Since many died soon thereafter, the data for only the first 4 days are included in Figure 4.

Figure 4A indicates that injections of 50 μl of blood from two-day-old virgin females did not stimulate oviposition. Similarly, injections of 50 μl of Ephrussi-Beadle Ringer's solution did not change the oviposition pattern.

Blood was drawn from females on the morning after mating was completed. Of 11 females injected with this "mated blood," nine began laying eggs at an increased rate as indicated in Figure 4B. By the third day the oviposition rate had returned to the virgin level (Table I). Surprisingly, injection of blood from females immediately after mating (about 12 hours after the beginning of mating) did not cause the switchover in behavior (Table I). Apparently, the circulating hormone(s) titer must increase with time after mating.

The blood volume of two two-day-old Cecropia females was measured by injecting 0.36 μg of inulin-methoxy-H^3. Inulin is neither excreted nor taken up by the cells of these insects (Cherbas and Cherbas, 1970). Equilibration appears to be complete in 4–6 hours in pupae (Cherbas and Cherbas, 1970); but to be certain, blood samples were taken 22 hours after the inulin injection. The blood volume of the 4.1 g and the 4.25 g females was found to be 1.1 and 1.4 ml $\pm$ 10%, respectively. Thus, the 50 μl hemolymph injections were about 4% of the total hemolymph volume.

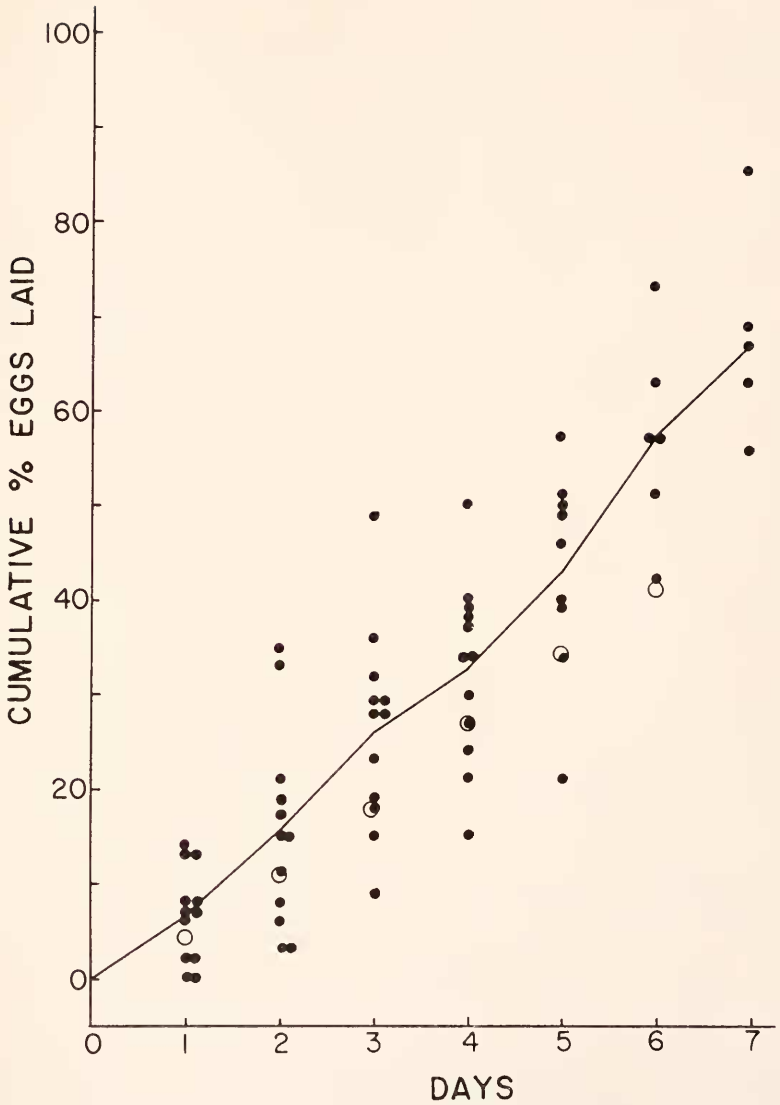

FIGURE 3. The oviposition pattern of virgin Cecropia females, each of which has received an implant of a bursa copulatrix from a female mated to a castrate male. The abscissa refers to days after implantation. The line is drawn through the average of the closed circles. The open circles represent the average cumulative percentage of eggs laid by females after mating to castrate males (Truman and Riddiford, 1971).

DISCUSSION

In the Lepidoptera, mating has long been known to stimulate oogenesis and increase the rate of oviposition (Klatt, 1920; Norris, 1933; Mokia, 1941; Benz, 1969; Truman and Riddiford, 1971). In all these cases the presence of sperm in

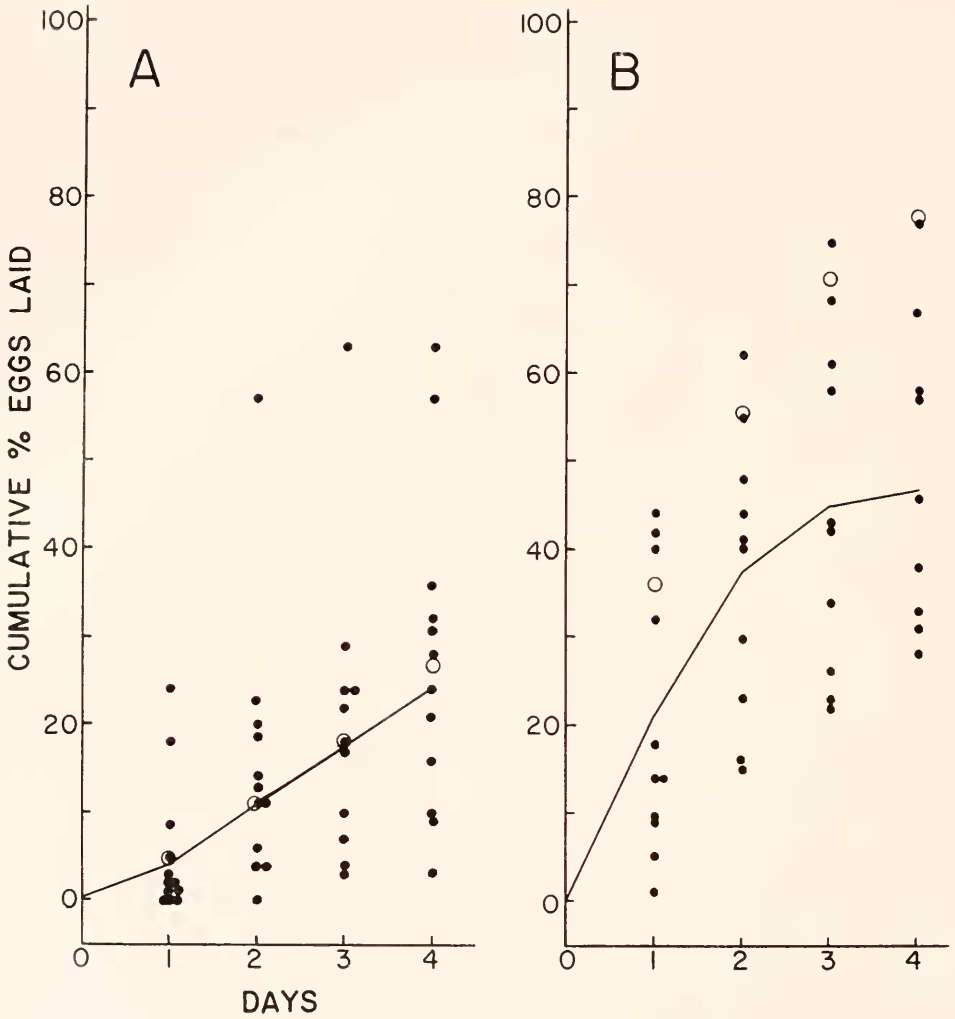

FIGURE 4. The oviposition pattern of virgin Cecropia females, each of which has received 50 μl of hemolymph. The abscissa refer to days after implantation. The line is drawn through the average of the closed circles excluding the highest set in Figure 4A: (A.) virgin hemolymph; (B.) mated hemolymph. The open circles represent the average cumulative percentage oviposition by intact virgin and mated females, respectively (Truman and Riddiford, 1971).

the female reproductive tract appears to be necessary for this switchover. It has been assumed that the presence of sperm in the spermatheca (Norris, 1933; Mokia, 1941; Benz, 1969) is the trigger just as has been found in the hemipteran *Rhodnius prolixus* (Davey, 1965). But the results reported above show that, at least in the non-feeding saturniid moth, *H. cecropia,* the important step is probably an interaction between the sperm and the bursa copulatrix. The male accessory gland

TABLE 1

Average daily oviposition of virgin female Cecropia moths after hemolymph injections

Treatment	Number	Average % eggs laid per day						
		Day after Treatment						
		1*	2	3	4	5	6	7
Untreated virgin females**	12	5	7	7	9	7	7	—
Untreated mated females**	24	36	22	13	7	6	3	—
Virgin females injected with blood from virgin females	12	4	10	6	6	10	11	8
Virgin females injected with blood from mated females 12 hours after the beginning of mating	3	1	4	3	5	10	16	16
Virgin females injected with blood from mated females the morning after mating	11	21	16	12	5	6	5	14

* Day 1 refers to day 3 of life. Day 3 is the day when most normal virgin females begin to lay a few eggs and is also the day following injection.

** From data of Truman and Riddiford (1971).

secretions which comprise the spermatophore do not trigger this response as indicated by the fact that implanted bursae of females mated to castrate males do not cause a change in oviposition rate. We have not ruled out, however, the possibility that, besides producing sperm, the testis also makes another substance which is responsible for activating the bursa.

After a fertile mating the bursa secretes a substance into the blood which causes a switchover from virgin to mated behavior. Secretory cells have been observed in the distal bursa copulatrix of several female Lepidoptera (Klatt, 1920; Weidner, 1934). The function of these cells is unknown, although Weidner (1934) proposes that the secretion may prevent the re-entry of the sperm into the bursa after they have emigrated to the spermatheca. Omura (1938) observed secretory cells in the anterior portion of the ductus bursae in the silkmoth *Bombyx mori,* but ascribes no function to them. Possibly one of these secretions is responsible for triggering the mated response.

This bursa factor does not stimulate directly an increase in oviposition, since mated cardiaectomized-allatectomized females lay eggs in the virgin pattern (Truman and Riddiford, 1971). Instead, this substance must act either directly or indirectly on the intrinsic cells of the corpora cardiaca to shut off the release of the calling hormone and to turn on the release of the oviposition stimulating hormone (Truman and Riddiford, 1971). Additional support for this role of the bursa factor is afforded by the observation that virgin females receiving bursa implants from mated females displayed other behaviors which were typical of mated females—they never "called" and they showed enhanced flight activity (Truman, Lounibos, and Riddiford, unpublished observations). Since intact nervous con-

nections between the brain and corpora cardiaca are necessary for this release (Truman and Riddiford, 1971), it seems probable that the bursa factor acts indirectly on the corpora cardiaca. The exact mode of action is presently unknown.

Moika (1941) showed that injection of blood from mated *B. mori* females induced oviposition in virgin females. Similiarly, in Cecropia mated hemolymph provoked an increase in oviposition rate over that of the normal virgin. But it should be noted that the response was transient and did not reach the mated level. This lack of a complete response is not surprising since the volume of hemolymph injected was only about 4% of the total blood volume. The absence of a continuing source of hormone as is present in the hosts receiving bursa implants from mated females is likely responsible for the swift decay back to the virgin level.

The bursa factor does not act directly to stimulate oviposition, but rather causes the corpora cardiaca to secrete an oviposition-stimulating hormone. Thus, hemolymph obtained from mated females may contain either or both the factor from the bursa and the hormone from the corpora cardiaca.

In Cecropia the spermatophore is completely formed in the bursa copulatrix by 3 hours and sperm transfer to the spermatheca occurs for the next 6 to 10 hours (J. A. Shepherd, Harvard University, personal communication). Thus, any crucial interaction of the sperm with the bursa must occur by 13 hours. The fact that the bursa is fully competent by 12 hours after the onset of mating to cause the change to the mated oviposition behavior is consistent with this hypothesis. As noted above, Weidner (1934) reported that during sperm transfer a secretion appears in the bursa. Possibly the release of the bursa factor into the blood also begins at this time.

Yet the blood taken from females 12 hours after the onset of mating lacks oviposition-stimulating activity. Normally, a mated Cecropia female under our 17L:7D photoperiod conditions begins oviposition about 17 to 18 hours after the initiation of mating (*i.e.* at or soon after the lights-off signal). Consequently, the corpora cardiaca probably release their oviposition-stimulating hormone about this time. Thus, 6 hours earlier, the oviposition-stimulating hormone was probably absent from the blood and the titer of the bursa factor was apparently not high enough to stimulate the corpora cardiaca of an injected host. By the following morning a sufficient titer of one or both hormones was present to stimulate oviposition.

The female Cecropia moth has a finely tuned hormonal control over her reproductive behavior. As a virgin female she releases her sex pheromone at a specific time of day determined by photoperiod (Riddiford and Williams, 1971). Once mated, pheromone release ceases and eggs are laid at an increased rate (Truman and Riddiford, 1971). The cue for this switchover is an interaction of sperm or substances from the testes with the bursa copulatrix. This interaction somehow causes the secretion of a factor from the bursa which in turn stimulates the corpora cardiaca to secrete the oviposition-stimulating hormone.

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Summary

1. Mating greatly increases the oviposition rate of female Cecropia silkmoths.

2. Implantation of the spermatheca from a mated female (either with or without sperm) into a virgin female did not alter the typical virgin oviposition pattern.

3. After implantation of the bursa copulatrix (minus the spermatophore) from a mated female, the virgin female oviposited eggs in the typical mated pattern.

4. Similar implantations of bursae from virgin females or from females which had mated with castrate males did not alter the virgin oviposition pattern.

5. Injections of hemolymph from mated females into virgin females caused an increase in oviposition rate. Blood from virgin females had no effect on oviposition.

6. Thus, the change in oviposition upon mating is due to a blood-borne factor which is secreted by the bursa copulatrix after contact with sperm or other substance from the testis. This bursa factor most probably acts to trigger the release of the oviposition-stimulating hormone from the intrinsic cells of the corpora cardiaca.

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